



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

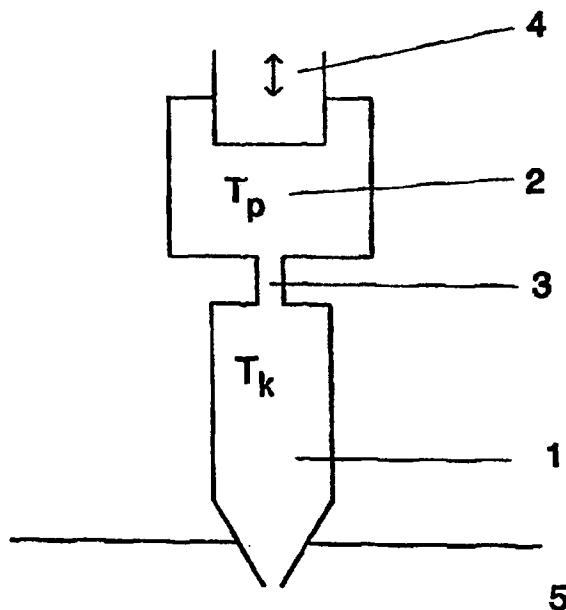
(51) International Patent Classification ⁶ : B01L 3/02, G01N 35/10 // G01F 15/02	A1	(11) International Publication Number: WO 97/02893						
		(43) International Publication Date: 30 January 1997 (30.01.97)						
<p>(21) International Application Number: PCT/FI96/00398</p> <p>(22) International Filing Date: 5 July 1996 (05.07.96)</p> <p>(30) Priority Data:</p> <table border="0"> <tr> <td>953344</td> <td>7 July 1995 (07.07.95)</td> <td>FI</td> </tr> <tr> <td>961337</td> <td>22 March 1996 (22.03.96)</td> <td>FI</td> </tr> </table> <p>(71) Applicant (for all designated States except US): BIOHIT OY [FI/FI]; Verkkosaarencatu 4, FIN-00580 Helsinki (FI).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): HARJUNMAA, Hannu [FI/US]; 14 Damon Road, Holden, MA 01520 (US). SUOVANIEMI, Osmo [FI/FI]; Kulopolku 6, FIN-00570 Helsinki (FI).</p> <p>(74) Agent: OY JALO ANT-WUORINEN AB; Iso Roobertinkatu 4-6 A, FIN-00120 Helsinki (FI).</p>		953344	7 July 1995 (07.07.95)	FI	961337	22 March 1996 (22.03.96)	FI	<p>(81) Designated States: AT, AU, CA, CZ, DE, ES, FI, GB, HU, IL, IS, JP, KR, LU, NO, NZ, PL, PT, RU, SE, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report.</i></p>
953344	7 July 1995 (07.07.95)	FI						
961337	22 March 1996 (22.03.96)	FI						

(54) Title: METHOD FOR CORRECTING A LIQUID DISPENSING ERROR, AND A LIQUID DISPENSING DEVICE

(57) Abstract

The object of the invention is a method for correcting a temperature dependent dispensing error occurring during liquid dispensing, such as pipetting, as well as a liquid dispensing device, such as a pipette, having a higher degree of accuracy. According to the method the dispensing takes place by means of two chambers (1, 2) connected to each other over a gas passage, wherein the first chamber (1) communicates, in addition to the said gas passage, with the liquid (5) to be dispensed, and the second chamber (2) is gas tight except for the said gas passage (3), whereby, in order to receive liquid in the dispensing device, the volume of the second chamber is increased, resulting in gas flowing therein from the first chamber, and in turn, liquid to be dispensed flowing into the first chamber until a pressure equilibrium between the chambers has been reached. According to the invention the change in temperature of the gas flowing from the first chamber to the second

is measured, and the change in volume effected in the second chamber is corrected based on the said measured temperature change so that the desired quantity of liquid is received in the first chamber. For the temperature measurement, a temperature sensor (7) mounted in the second chamber (2) in the vicinity of the gas passage (3) and in addition, possibly also a sensor (6) mounted in the first chamber (1) is used.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

Method for correcting a liquid dispensing error, and a liquid dispensing device

5 The object of the present invention is a method for eliminating a temperature dependant dispensing error in connection with the dispensing of liquids.

Specifically the invention is used in connection with a dispensing method, wherein the dispensing takes place by means of two chambers connected to each other over a gas passage, wherein the first chamber communicates, in addition to the said gas passage, with the liquid to be dispensed, and the second chamber is gas tight except for the said gas passage, whereby, in order to take up liquid in the dispensing device, the volume of the second chamber is increased, resulting in gas flowing therein from the first chamber, and in turn, liquid to be dispensed flowing into the first chamber until a pressure equilibrium between the chambers has been reached.

20 The object of the invention is also a liquid dispensing device comprising two chambers interconnected over a gas passage, the first chamber communicating, in addition to the said gas passage, with the liquid to be dispensed, and the second chamber being gas tight except for the said gas passage, and containing means for regulating the volume thereof.

30 The method according to the invention is applied typically, but not solely, when pipetting liquids using an electronic or mechanical pipette, and it allows for the elimination of or at least a substantial reduction of a liquid dispensing error resulting from temperature differences between the sample and the pipette.

35 In laboratory analyses pipetting and dispensing of samples and reagents which are usually in liquid form comprises the most difficult stage in the process of ob-

taining an accuracy of, for example, less than half a percent in accuracy as well as in reproducibility. The pipetting accuracy using the more old-fashioned technique has usually been in the magnitude of one microlitre (with an inaccuracy of even 5 to 10 %, especially at smaller volumes), if not worse (Rodgers et al, Clin. Chem. 20/1, 43-50, 1974 and Pardue et al, Clin. Chem. 20/8, 1028-1042, 1974). Using the modern techniques of today, the accuracy of both mechanical and electronic pipettes varies in the range of 0.1 - 5 % (Suovaniemi: Dissertati-on, ISBN 952-90-5248-0, University of Helsinki, 1994).

The pipetting inaccuracy depends on both the apparatus and also its user. The effect of human error is more often evident especially in connection with the use of mechanical pipettes as compared to the use of electronic pipettes. The quality of the pipette tip and the manner of its use has its own small effect, except when the tip is placed on to the pipette by hand. In this case the tip may become contaminated or compressed so that the tip regains its shape during the first pipetting operations and in connection therewith draws an excess liquid into the tip. This error can be eliminated e.g. by using vertically packaged tips or by handling the tips with extreme care without changing their form when mounting them on a pipette. The use of an electronic pipette removes the human pipetting errors, as well as the use of automatic calibration of an electronic pipette in connection with each use (Hattori, Super Pipetter Seminar: Standardized Risk Factor and Support to Validation, Biohit, 1994). Improving these factors usually leads to pipetting results which are only 0.1 - 0.5 % more accurate.

According to the invention a method is now provided for eliminating or at least reducing substantially temperature dependent dispensing errors occurring in liquid dispensing methods based on the air-displacement principle.

The object of the invention is achieved by a method and device according to the appended claims.

Specifically, the method according to the invention is characterized in that the change in temperature of the gas flowing from the first chamber to the second is measured, and the change or increase in volume caused in the second chamber for taking up liquid, is corrected based on the said measured temperature change so that the desired quantity of liquid is received in the first chamber.

According to the invention the said temperature change is measured prior to liquid sampling with temperature sensors suitably located in each chamber, whereby one measures the temperature of the gas in the first chamber, and the second measures the temperature prevailing in the second chamber, that is the temperature which the gas flowing from one chamber to the other during liquid sampling reaches. The difference in these temperatures corresponds to the temperature change of the gas flowing from the first chamber to the second.

According to a second embodiment of the invention the method can be carried out using only one sensor, which is placed in the second chamber. This sensor measures first the temperature of the second chamber prior to liquid sampling, and a second time, during liquid sampling, the temperature of the gas flowing from the first chamber to the second chamber, the difference being the said change in temperature.

An object of the invention is also a liquid dispensing device of the afore mentioned type which is characterized in that a temperature sensor is provided for measuring the change in the temperature of the gas flowing from one chamber to the other via the gas passage, and means for feeding a corresponding correction command to the volume

regulating means in the second chamber.

According to the invention, both chambers can contain a sensor, or alternatively only the second chamber contains
5 a sensor.

In the following, the invention is described as applied to liquid dispensing by pipetting and an electrical pipette, but the invention is not limited to pipettes, but
10 it can be applied in a variety of liquid dispensing systems within the scope of the appended claims.

The invention is based on the observation that the quantity of air which in the drawing stage of pipetting flows
15 from the pipette tip to the pipette body quickly attains the temperature of the pipette body. The change in the volume of the air space in the body which is caused by moving the plunger of the pipette in order to draw liquid into the pipette tip, is equal to the selected pipetting
20 volume, i.e. the pipette set value. As a result of the plunger movement, the pressure in the body decreases, and air flows from the tip into the body until the pressures are substantially in balance again. In case the temperatures of the air space in the tip and the pipette body
25 are not equal, the final volume of the quantity of air moved is, after evening out of the temperatures, different in the pipette body from the volume that was intended to be removed from the tip. The difference between the nominal and real volumes of the quantity of air
30 transferred, is also the volumetric error of the liquid received in the tip.

The evening out of the temperature in the pipette body takes place rapidly as the air passage there is relative-
35 ly narrow, i.e. the distance of all the air molecules from the closest wall is small, usually less than 1 mm. In addition the specific heat capacity of air is small

compared to the specific heat capacity of the material of the body. The air entering the body attains the temperature of the body before the tip has been lifted out of the sample, wherefore the changed volume of the air transferred affects directly the pipetting result. In the beginning and the end of pipetting the pressures inside and outside the pipette are substantially in equilibrium, and any transient pressure differences do not affect the end result.

10

An important consequence of this observation is that there is no need to measure the temperature of the liquid. Decisive is the temperature of the air space from which air moves to the pipette body, and specifically the temperature difference between that air space and the body. After a few pipettings, the air space within the tip reaches substantially the temperature of the liquid, but measuring the temperature of the air space in the tip makes a correction possible which is correct from the beginning on. It is also to be observed that the correction according to this invention also eliminates any error resulting from a deviating tip temperature. Such a situation is present, for example, when applying a tip with warm fingers.

25

In the following a correction formula is derived which has to be used by the processor in connection with an electrical pipette, for it to be able to change the movement of the plunger from its set value so that the quantity of liquid to be drawn is of the desired magnitude in all temperature conditions.

30

The formula for calculation is derived from Gay-Lussac's law which stipulates that at constant pressure the volume of a gas is proportional to its absolute temperature. It is assumed that the absolute temperature of the pipette body is T_p and the absolute temperature of the air space

35

of the tip is T_k (see also Fig. 1). The nominal pipetting volume, i.e. the set value, is V . In the initial stage the tip is immersed in the liquid, but not so deeply that the hydrostatic pressure is capable of substantially forcing liquid into the tip. Now the plunger is drawn back to a degree corresponding to the volume V and the pressure inside the pipette body decreases. As there is a tendency to re-establish the pressure equilibrium, air at a temperature of T_k in a volumetric amount V flows from the tip into the pipette body. This quantity of air is now subjected to the temperature T_p . In other words, the new volume V' of the air transferred is according to the Gay-Lussac's law

$$V' = (T_p/T_k) V.$$

As pressure equilibrium has to be established at the end, a further volume of air

$$\Delta V = V - V' = (1 - T_p/T_k) V.$$

has to transfer between the tip and the body. As this air comes from the tip, due to the pressure equilibrium, it has to be replaced by an equal volume of liquid, and the pipetting error is ΔV . It is positive (too big a pipetting result) if $T_k > T_p$, and negative (too small a pipetting result) if $T_k < T_p$. In the positive results, there is an additional small excess as also ΔV is subjected to the temperature T_p . This is of no practical consequence, but can easily be taken into account, if desired.

The actual pipetting result V'' as a function of the nominal volume (the movement of the plunger) V and the temperatures is consequently

$$V'' = V + \Delta V = (2 - T_p/T_k) V.$$

The correction can be implemented in an electronic pipette in a straightforward manner. The temperatures can be read continuously at specific short intervals, or only when pushing the pipetting button, and according to these and the nominal volume, an activation command corresponding to V_k (the corrected volume) is given to the plunger:

$$V_k = V / (2 - T_p / T_k).$$

In the following, reference is made to the appended drawing where Figure 1 shows in a general and schematic manner a liquid dispensing device according to the invention, the Figure 2 shows in a schematic manner a pipette according to the invention for carrying out the method, and Figure 3 a constructional and mounting system for a sensor for measuring the temperature.

In the Figure 1, two gas filled chambers for receiving liquid is shown, a first chamber 1 and a second chamber 2, which communicate over a gas passage 3, and the interior pressure of which is substantially in balance at the beginning and the end of liquid sampling. The volume of the second chamber 2 can be enlarged in order to receive liquid by suitable means, for example by means of a reciprocating plunger 4 mounted in a gas-tight manner in the chamber 2. The first chamber 1 can be made to communicate with the liquid 5. As a result of the pressure difference brought about by the movement of the plunger 4, liquid flows into the chamber 1 until a pressure equilibrium is essentially established. The accurate liquid volume received in the chamber 1 is thereafter dispensed in one or more further dishes. In the system, a quantity of gas thus moves from one chamber to the other, which quantity corresponds to the liquid volume to be drawn, the volume of which, however, during drawing of the liquid or thereafter changes in case the temperature of the chambers is not uniform. If this change in gas volume cannot be

taken into account, a dispensing error results.

The Figure 2 shows a pipette, which comprises a cylinder space forming the air space 9 of the pipette, the cylinder space having a gas connection to the pipette tip 8, a reciprocating plunger 4 in the cylinder space, as well not further illustrated operating means for the plunger. According to a preferred embodiment of the invention, the pipette comprises sensors 6 and 7 for measuring the temperature. A suitable means for measuring the temperature is, for example, a NTC resistance (Negative Temperature Coefficient resistance) functioning as a temperature sensor. Such resistances are known per se and commercially available. According to this embodiment, the temperature of the gas is measured by the sensors 6 and 7 in the tip 8 and the air space 9 before liquid sampling, and the temperature difference measured is used for feeding a correction command to the operating means for moving the plunger in accordance with the formula derived earlier.

20

In the Figure 3 a favourable embodiment for attaching a sensor to the dispensing device, for example a pipette body, is shown. In the Figure 3, there is disclosed on an enlarged scale a interchangeable tip 8, into which the lower part of the pipette body projects with its gas passage 3 extending into the air space 9 of the pipette. The sensor 6 is fastened to a handle-like device 10 opening towards the body, and attached between the legs of the handle, the sensor 6 extending into the air space of the tip itself. The handle-like device 10 functions, besides as a fastening means, also as a means for protecting the sensor. It is self-evident that also the sensor 7 in the air space can be constructed in a manner corresponding to the embodiment shown. The device 10 has to be dimensioned so that neither it, nor the sensor 6, is brought into contact with the liquid to be dispensed.

35

Claims

1. Method for correcting a temperature dependant dispensing error occurring during dispensing of a liquid, wherein the dispensing takes place by means of two chambers connected to each other over a gas passage, wherein the first chamber communicates, in addition to the said gas passage, with the liquid to be dispensed, and the second chamber is gas tight except for the said gas passage, whereby, in order to take up liquid in the dispensing device, the volume of the second chamber is increased, resulting in gas flowing therein from the first chamber, and in turn, liquid to be dispensed flowing into the first chamber until a pressure equilibrium between the chambers has been reached, characterized in that the change in temperature of the gas flowing from the first chamber to the second is measured, and the change in volume caused in the second chamber is corrected based on the said measured temperature change so that the desired quantity of liquid is received in the first chamber.
2. The method according to claim 1, characterized in that the interior temperature of both chambers is measured prior to liquid sampling by means of sensors mounted separately in each chamber.
3. The method according to claim 1, characterized in that for measuring the change in the gas temperature, a sensor placed in the second chamber is used, which prior to liquid sampling measures the interior temperature of the said chamber and during liquid sampling measures the temperature of the gas flowing from the first chamber to the second.
4. Liquid dispensing device comprising two chambers (1,2) interconnected over a gas passage, the first chamber (1)

communicating, in addition to the said gas passage (3), with the liquid (5) to be dispensed, and the second chamber (2) being gas tight except for the said gas passage, and containing means (4) for regulating the volume thereof, **characterized** in a temperature sensor (6, 7) being provided for measuring the change in the temperature of the gas transferring from one chamber to the other, and means for feeding a corresponding correction command to the volume regulating means (4) in the second chamber (2).

5. The liquid dispensing device according to claim 4, **characterized** in that both chambers (1, 2) contain a temperature sensor (6, 7).

6. The liquid dispensing device according to claim 4, **characterized** in that only the second chamber (2) contains a temperature sensor (7).

7. The liquid dispensing device according to any one of the claims 4-6, **characterized** in that the temperature sensors are NTC-sensors.

8. The liquid dispensing device according to any one of the claims 4-7, **characterized** in that the means for regulating the volume in the second chamber is a plunger mounted gas-tightly in the chamber.

9. The liquid dispensing device according to any one of the claims 4-8, **characterized** in that the first chamber is a pipette tip (8).

10. The liquid dispensing device according to claim 7, wherein the first chamber is comprised of a preferably detachable pipette tip (8), and the second chamber is comprised of an air space (9) formed in a pipette body, which communicates with the pipette tip by means of a

passage (3) through the body, **characterized** in that the sensor is over a mounting device (10) fastened to the body, in the close vicinity to the gas passage between the tip and the air space.

5

11. The liquid dispensing device according to claim 10, **characterized** in that the mounting device (10) is handle-like and the sensor is mounted between the legs of the handle, the ends of the legs being attached to the body
10 of the device.

1/2

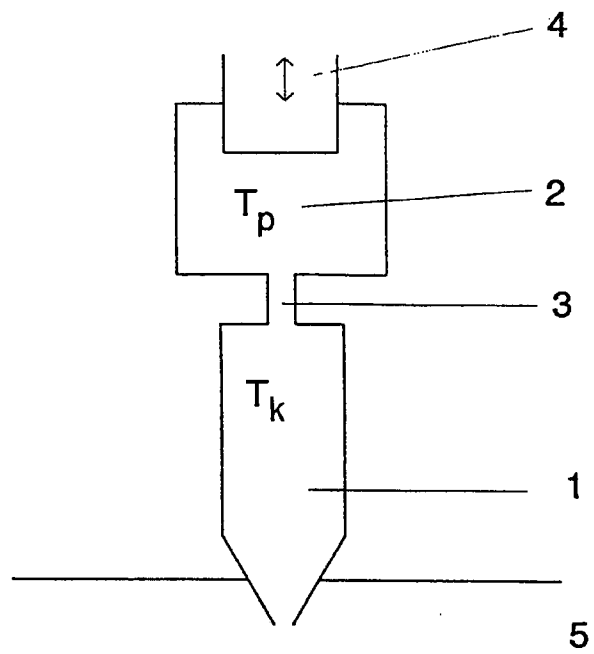


Fig. 1

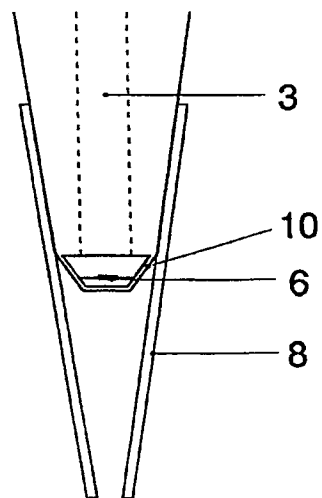


Fig. 3

2/2

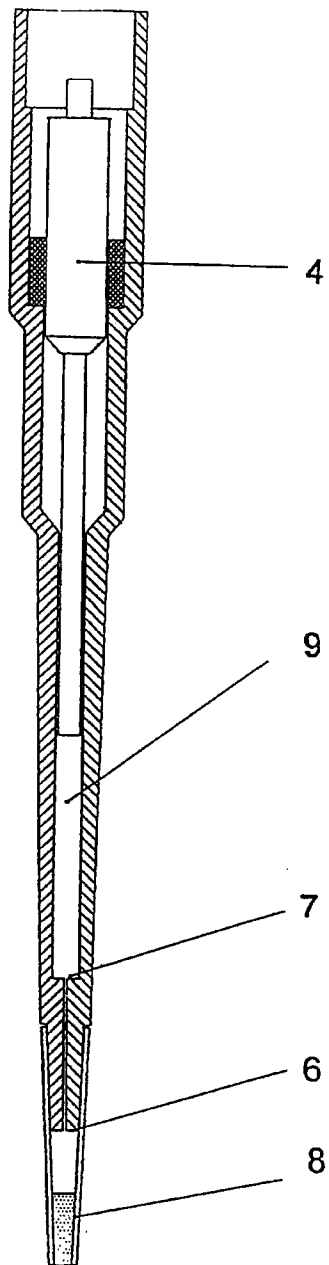


Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 96/00398

A. CLASSIFICATION OF SUBJECT MATTER		
IPC6: B01L 3/02, G01N 35/10 // G01F 15/02 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC6: B01L, G01N, G01F		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
SE,DK,FI,NO classes as above		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
WPI, EPODOC		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0337460 A2 (PRZEDSIĘBIORSTWO POLONIJO ZAGRANICZNE PLASTOMED), 18 October 1989 (18.10.89), column 3, line 2 - column 5, line 12, figures 1,2 --	1-11
A	US 5187990 A (MAGNUSSEN, JR HAAKON T. ET AL), 23 February 1993 (23.02.93), column 18, line 23 - column 19, line 51, abstract --	1-11
A	US 5287758 A (MANFRED GEISS ET AL), 22 February 1994 (22.02.94), column 2, line 27 - line 40, abstract -- -----	1-11
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
4 October 1996		10 -10- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86		Authorized officer Gunnel Wästerlid Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT
Information on patent family members

05/09/96

International application No.

PCT/FI 96/00398

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0337460	18/10/89	NONE	
US-A- 5187990	23/02/93	US-A- 4905526	06/03/90
		AU-B- 589891	26/10/89
		AU-A- 3890685	22/08/85
		CA-A- 1293709	31/12/91
		DE-A,T- 3586289	13/08/92
		DE-D,T- 3588071	13/06/96
		EP-A,B- 0152120	21/08/85
		EP-A,B- 0428500	22/05/91
		FR-A- 2559904	23/08/85
		JP-C- 1807271	10/12/93
		JP-B- 5013709	23/02/93
		JP-A- 60193549	02/10/85
		US-A- 4671123	09/06/87
US-A- 5287758	22/02/94	AU-B- 654995	01/12/94
		AU-A- 1045592	30/07/92
		DE-A- 4102336	06/08/92
		DE-U- 9111441	09/01/92
		JP-A- 6063424	08/03/94